Deletion in the first cysteine-rich repeat of low density lipoprotein receptor impairs its transport but not lipoprotein binding in fibroblasts from a subject with familial hypercholesterolemia

(cholesterol metabolism/human genetics/polymerase chain reaction)

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ABSTRACT   The ligand-binding domain of the low density lipoprotein (LDL) receptor is composed of seven cysteine-rich repeats, each ≈40 amino acids long. Previous studies by van Driel et al. [van Driel, I. R., Goldstein, J. L., Sudhof, T. C. & Brown, M. S. (1987) J. Biol. Chem. 262, 17443–17449] showed that if the first repeat of the ligand-binding domain (encoded by exon 2) is deleted, the receptor fails to bind an anti-LDL receptor monoclonal antibody (IgG-C7) but continues to bind LDL with high affinity. Cultured fibroblasts from a Black South African Xhosa patient (TT) with the clinical syndrome of homozygous familial hypercholesterolemia demonstrated high-affinity cell-surface binding of ^{125}I-labeled LDL but not ^{125}I-labeled IgG-C7. Previous haplotype analysis, using 10 restriction fragment length polymorphic sites, suggested that the patient inherited two identical LDL receptor alleles. The polymerase chain reaction technique was used to selectively amplify exon 2 of the LDL receptor gene from this patient. Sequence analysis of the amplified fragment disclosed a deletion of six base pairs that removes two amino acids, aspartic acid and glycine, from the first cysteine-rich ligand binding repeat. The mutation creates a new Pst I restriction site that can be used to detect the deletion. The existence of this mutant allele confirms that the epitope of IgG-C7 is located in the first cysteine-rich repeat and that this repeat is not necessary for LDL binding. The mutant gene produced a normally sized 120-kilodalton LDL receptor precursor protein that matured to the 160-kilodalton form at less than one-fourth the normal rate. Thus, deletion of two amino acids within the first cysteine-rich repeat retards receptor transport from the endoplasmic reticulum to the cell surface, in contrast to deletion of the entire first repeat, which has no effect on receptor maturation.

Mutations in the low density lipoprotein (LDL) receptor gene result in the autosomal dominant disease familial hypercholesterolemia (FH) (1). The LDL receptor is a transmembrane protein that binds LDL and mediates its uptake into the cell. Individuals with two mutant LDL receptor alleles have profoundly fewer or no functional LDL receptors on their cell surfaces and this is associated with markedly elevated plasma LDL–cholesterol levels. These individuals develop premature coronary atherosclerosis that often leads to myocardial infarctions in early childhood. In homozygous FH, mutations in each LDL receptor allele can be either identical (a true homozygote) or different (a compound heterozygote).

Numerous mutant LDL receptor alleles from patients with FH have been classified according to the phenotype of the LDL receptor protein produced. IgG-C7, a monoclonal antibody directed against the LDL receptor protein, has been used to analyze the synthesis of the LDL receptor in fibroblasts derived from skin biopsies of patients with FH (2). Four classes of mutations have been found (3). The normal LDL receptor protein is synthesized as a precursor with an apparent molecular mass of 120 kilodaltons (kDa). It contains 839 amino acids, unprocessed N-linked sugars, and nascent O-linked sugar chains. Within 30 min, this precursor is transported from the endoplasmic reticulum (ER) to the Golgi complex where high-mannose chains on the N-linked sugars are trimmed and galactose and sialic acid residues are added to the O-linked sugar chains (4). The apparent molecular mass of the protein increases from 120 kDa to 160 kDa. Subsequently, the receptor is transported from the Golgi complex to the cell surface where it is localized in coated pits. One class of mutations, the class II mutations, results in retardation or complete inhibition of the transport and processing of the LDL receptor protein. The 120-kDa precursor protein remains sequestered in the ER (3). Some patients have a "leaky" mutation in which the 120-kDa precursor protein predominates but a certain percentage traverses through the Golgi complex to the cell surface (3, 5).

The LDL receptor gene is composed of 18 exons (6). The protein has been divided into six different functional domains (6). The ligand-binding domain, encoded by exons 2–7, is composed of seven repeats, each ≈40 amino acids long. Each exon encodes a single repeat except for exon 4, which codes for three repeats. In a previous study using site-directed mutagenesis, van Driel et al. (7) demonstrated that deletion of exon 2, which codes for the first cysteine-rich repeat, creates a protein that binds LDL with high affinity but does not bind IgG-C7, a monoclonal antibody specific for the first repeat (7).

Cell surface binding studies of fibroblasts from a Black South African subject with homozygous FH (TT) showed binding characteristics that were similar to those of the previously characterized mutant receptor lacking exon 2. The cells from TT failed to bind ^{125}I-labeled (^{125}I-) IgG-C7 but bound ^{125}I-LDL with high affinity, though in decreased amounts. Therefore, exon 2 was suspected to be the site of LDL receptor mutation in this FH homozygote. To test this hypothesis, we used the polymerase chain reaction (PCR) technique to amplify and sequence exon 2 from patient TT. This technique, which was developed by Saiki et al. (8), has previously been used to characterize mutations at the β-globin locus by amplification of genomic DNA and direct sequencing (9). Our current results disclose an in-frame

deletion of 6 base pairs (bp) that destroys the structural integrity of the first repeat in the LDL receptor.

METHODS

Materials. Human LDL (density, 1.019–1.063 g/ml) and lipoprotein-deficient serum (density, >1.25 g/ml) were prepared as described (10). The LDL was radioiodinated with ^{125}I by the iodine monochloride method (10). Tran^{35}S-label (70% [^{35}S]methionine) was obtained from ICN, and 25-hydroxycholesterol from Research Plus (Bayonne, NJ). IgG-C7, a mouse monoclonal antibody that recognizes an epitope in the first cysteine-rich repeat of the binding domain of the human LDL receptor (7), was prepared and radiolabeled with ^{125}I as described (2). A polyclonal anti-LDL receptor antibody, 698-7, was raised against purified bovine LDL receptor (11). B3/25, a mouse monoclonal antibody to the human transferrin receptor, was provided by I. S. Trowbridge (The Salk Institute for Biological Studies, San Diego, CA). Oligonucleotides were synthesized on an Applied Biosystems (Foster City, CA) DNA synthesizer. [γ-^{32}P]ATP was purchased from ICN. *Thermus aquaticus* DNA polymerase was purchased from Perkin–Elmer Cetus (Norwalk, CT) and *Pst* I restriction endonuclease was purchased from New England Biolabs.

LDL Receptor Assays. Fibroblasts were obtained from skin biopsy specimens and grown in monolayer culture (10). The binding, internalization, and degradation of ^{125}I-LDL at 37°C and the binding of ^{125}I-LDL at 4°C were assessed as described (12). Surface binding of ^{125}I-IgG-C7 at 4°C and internalization and degradation of the same antibody at 37°C were also measured (10). Nonlinear regression analysis using the program Enzfitter (Elsevier/BIOSOFT, Cambridge, U.K.) was used to calculate the high-affinity components of the data curves, assuming ligand binding to a single class of high-affinity sites.

Immunochemical Analysis of [^{35}S]Methionine-Labeled LDL Receptors. Monolayers of fibroblasts were incubated with [^{35}S]methionine as described (13). After labeling, cells were incubated in medium containing 200 μM methionine and harvested after various time intervals. The cells were washed and solubilized prior to immunoprecipitation using preformed immune complexes containing either the monoclonal anti-LDL receptor antibody IgG-C7 or the polyclonal anti-LDL receptor antibody 698-7 (13). Immunoprecipitates were subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis (5–20% acrylamide slab gels). Gels were enhanced with salicylate (14), dried, and subjected to fluorography.

Amplification of Genomic DNA. Genomic DNA was isolated from skin fibroblasts (15) and diluted to a final concentration of 0.1 μg/μl in buffer containing 1 mM Tris Cl (pH 8.0) and 1 mM EDTA. One microgram of the diluted DNA was subjected to gene amplification by the polymerase chain reaction (8) with the following modifications: (*i*) the DNA was annealed to the oligonucleotides at 68°C, (*ii*) extension was carried out at the same temperature for 6 min, and (*iii*) denaturation was performed at 95°C for 1 min. Two oligodeoxynucleotides complementary to the DNA sequences flanking exon 2 of the LDL receptor gene; PCR57 (5'-CCTTTCTCCTTTTCCTCTCTCTCAG-3') and PCR58 (5'-AAAATAAATGCATATCATGCCCAAA-3'), were used in the amplification reaction. In each reaction an unlabeled and a [γ-^{32}P]ATP end-labeled (16) oligonucleotide were used. The amplified DNA was subjected to electrophoresis in a 6% polyacrylamide gel in buffer A (0.09 M Tris/0.09 M boric acid/2 mM EDTA) at 200 V for 1 hr and then to autoradiography on Kodak XAR-5 film for 1 min. The amplified DNA was eluted from the gel, extracted twice with phenol/chloroform (1:1, vol/vol), and precipitated with 0.3 M sodium acetate (pH 5) and ethanol.

Sequence Analysis. Amplified, end-labeled fragment [$\geq$200,000 cpm (Cherenkov)] was used for sequence analysis according to the Maxam–Gilbert technique (17). Both strands of exon 2 were sequenced by using DNA that had been amplified in separate experiments.

Restriction Enzyme Digestion. Approximately 100 ng of amplified, end-labeled DNA (300,000 cpm) was subjected to restriction enzyme digestion with 20 units of *Pst* I. After digestion, the DNA was extracted with phenol/chloroform (1:1) and precipitated with 0.3 M sodium acetate (pH 5) and ethanol. Digested DNA was electrophoresed in a 6% polyacrylamide gel in buffer A at 200 V for 1 hr. The gel was then subjected to autoradiography on XAR-5 film for 5 min.

RESULTS

LDL Receptor Assays. The binding of ^{125}I-LDL and ^{125}I-IgG-C7 antibody at 4°C to fibroblast cultures from TT and from a normal subject were compared (Fig. 1). The cells were incubated with various concentrations of ^{125}I-LDL or ^{125}I-IgG-C7 until binding equilibrium was reached. Maximal high-affinity ^{125}I-LDL binding to TT cells (127 fmol/mg of protein) was 30% of the value of normal cells (380 fmol/mg of protein) (Fig. 1A). Nonlinear regression analysis showed K_d values for ^{125}I-LDL binding to be 1.0 ± 0.2 (mean ± SEM curve) and 1.6 ± 0.6 μg of protein per ml for normal and TT cells, respectively. Thus, the reduction in binding activity in fibroblasts from TT was due to a lower number of binding sites rather than a decrease in affinity for ^{125}I-LDL. In contrast to ^{125}I-LDL, ^{125}I-IgG-C7 failed to bind with high affinity to TT cells (Fig. 1B).

Fig. 2 compares the surface binding, internalization, and degradation of ^{125}I-LDL at 37°C in normal and TT cells. High-affinity surface binding of ^{125}I-LDL was about one-third the level of normal. The cells from TT internalized and degraded ^{125}I-LDL at rates commensurate with the reduced number of surface LDL receptors. At 37°C, these cells failed to show any specific binding, uptake, or degradation of ^{125}I-IgG-C7 (data not shown).

A polyclonal antibody to the bovine LDL receptor, 698-7, was used to immunoprecipitate the LDL receptor produced by TT and normal cells (Fig. 3). The fibroblasts were pulsed for 30 min with [^{35}S]methionine and then "chased" with unlabeled methionine for the indicated times. In normal fibroblasts most of the 120-kDa precursor was processed to the 160-kDa mature form after 30 min, and complete conversion occurred by 60 min (Fig. 3 *Left*). In the cells from TT, the 120-kDa precursor was seen after a 30-min pulse but the
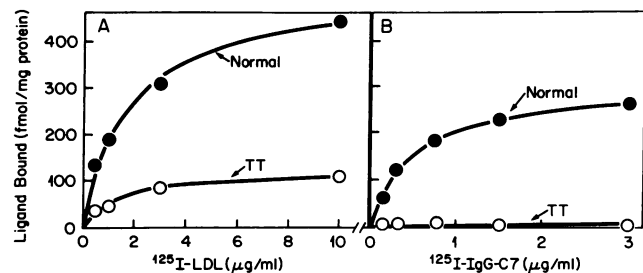

FIG. 1. Surface binding at 4°C of ^{125}I-LDL (*A*) and ^{125}I-IgG-C7 (*B*) to normal and TT fibroblasts. After incubation for 48 hr in lipoprotein-deficient medium, each monolayer received 1.5 ml of medium containing the indicated concentration of ^{125}I-LDL (299 cpm/ng of protein) or ^{125}I-IgG-C7 (1302 cpm/ng of protein). After 2 hr at 4°C the total radioactivity bound to the cells was determined in duplicate incubations for each point. The curves and data points represent high-affinity binding calculated by nonlinear regression analysis as described in *Methods*. Nonspecific (low-affinity) values comprised <5% of total values in the case of ^{125}I-LDL at 3 μg/ml and <20% of the total values for ^{125}I-IgG-C7 at 1.6 μg/ml in normal cells.
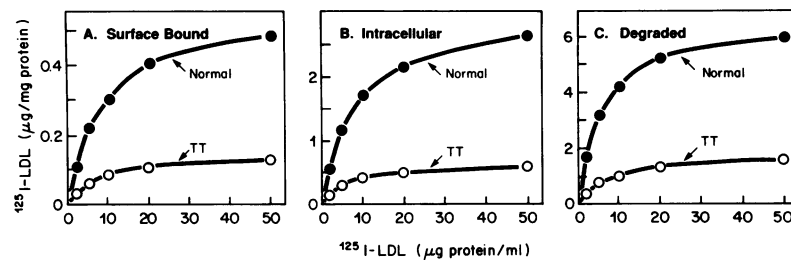
FIG. 2.   Surface binding (*A*), internalization (*B*), and degradation (*C*) of ^{125}I-LDL at 37°C in normal and TT cells. After incubation for 48 hr in lipoprotein-deficient medium, each monolayer received 2 ml of medium containing the indicated concentration of ^{125}I-LDL (600 cpm/ng of protein). After incubation for 4 hr at 37°C the amounts of surface-bound, intracellular, and degraded ^{125}I-LDL were determined in duplicate incubations for each value. The curves and data points represent high-affinity values calculated by nonlinear regression analysis. Nonspecific (low-affinity) values in all cases comprised <5% of total values in normal cells.

rate of its processing to the mature form was markedly delayed. Even after a 2-hr chase, approximately half of the receptor protein was still in the precursor form (Fig. 3 *Right*).

The synthesis and processing of LDL receptors in cells from the heterozygous mother of TT were analyzed by two sequential immunoprecipitations done after a pulse–chase protocol (Fig. 4). First, IgG-C7 was used to selectively precipitate the receptor produced from the normal allele; then, polyclonal 698-7 was used to precipitate the receptors produced by the mutant allele. As a control we immunoprecipitated the transferrin receptor by using B3/25, an antibody to the transferrin receptor. In cells from TT's mother and a normal subject, the first immunoprecipitation yielded 160- and 90-kDa bands that corresponded to the mature LDL and transferrin receptor, respectively. In TT's cells only the 90-kDa band was seen. We then used a polyclonal anti-LDL receptor antibody, 698-7, to immunoprecipitate receptor protein not recognized by IgG-C7 (Fig. 4). In cells from TT and his mother, but not in the normal cells, a large amount of the 120-kDa LDL receptor precursor protein was seen when the polyclonal antibody was used. With this antibody some mature 160-kDa receptor was also seen in cells from the normal subject and from TT's mother, due to incomplete prior immunoprecipitation with IgG-C7. Only a very small amount of the mature 160-kDa receptor was seen in TT cells, consistent with the slow processing of this mutant protein. These results show that the presence of the abnormal protein does not slow the processing of the normal protein in the heterozygous cells.

Sequence Analysis of TT's Receptor Allele. Genomic DNA from TT and a normal control was used to amplify exon 2 of the LDL receptor gene. End-labeled PCR-58 oligonucleotide
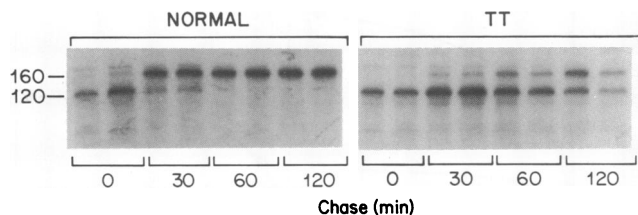
and unlabeled PCR-57 oligonucleotide were used for the amplification. These oligonucleotides corresponded to sequences in the introns immediately flanking exon 2 (see *Methods*). The nucleotide sequence of the amplified region was determined by the Maxam–Gilbert technique. The procedure revealed a 6-bp deletion in the TT gene (Fig. 5). Sequence analysis of the complementary strand of DNA amplified in a second experiment confirmed the deletion. The deletion eliminates two amino acids (aspartic acid-26 and glycine-27) from the first repeat of the LDL receptor protein (Fig. 6). The two amino acids deleted in TT are conserved between the normal human and rabbit LDL receptors. The significance of this will be addressed in *Discussion*.

Analysis of *Pst* I Restriction Site. The 6-bp deletion in TT creates a new *Pst*I restriction site in exon 2. Exon 2 of TT, his mother, and a normal control were selectively amplified by the polymerase chain reaction and were subsequently subjected to *Pst* I digestion. The restriction fragments were extracted, purified, and separated in a 6% polyacrylamide gel (Fig. 7). The undigested, amplified fragment is expected to be 183 bp long in the normal DNA and 177 bp long in the DNA from TT. In the normal gene there is no *Pst* I site in exon 2. Therefore, in this case only the full-length 183 bp fragment is seen after treatment with *Pst* I. *Pst* I digestion of amplified DNA from TT showed a 90-bp fragment, due to cleavage at the *Pst* I site that had been created by the 6-bp deletion. After *Pst* I digestion, the mother had both the normal 183-bp band and the mutant 90-bp fragment. (The father was not available
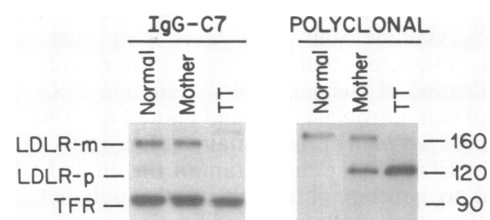

FIG. 3.   Biosynthesis of ^{35}S-labeled LDL receptors in fibroblasts from TT and a normal subject. After incubation for 16 hr in lipoprotein-deficient medium, cells were pulse-labeled for 30 min with [^{35}S]methionine (normal cells, 120 μCi/ml; cells from TT, 61 μCi/ml; 1 μCi = 37 kBq) in methionine-free Eagle's minimum essential medium and then incubated for the indicated time in medium containing 200 μM methionine to provide a chase. Cells were washed, detergent-extracted, and subjected to immunoprecipitation with the polyclonal anti-LDL receptor antibody 698-7. Immunoprecipitates were analyzed by NaDodSO₄/polyacrylamide gel electrophoresis and fluorography. Duplicate samples were analyzed. The positions of the mature 160-kDa LDL receptor bands and the precursor 120-kDa LDL receptor bands are indicated.

FIG. 4.   Biosynthesis of ^{35}S-labeled LDL receptors in fibroblasts from TT, his mother, and a normal subject. After incubation for 16 hr in lipoprotein-deficient medium, cells were pulse-labeled for 30 min with [^{35}S]methionine (70 μCi/ml) in methionine-free Eagle's minimum essential medium. Labeling was followed by a chase for 30 min in medium containing 200 μM methionine. (*Left*) Cells were washed, detergent-extracted, and subjected to immunoprecipitation with the monoclonal anti-LDL receptor antibody IgG-C7 and a monoclonal anti-transferrin receptor antibody. The precipitates were collected and analyzed by NaDodSO₄/polyacrylamide gel electrophoresis and fluorography. The positions of the mature 160-kDa LDL receptor (LDLR-m), the 120-kDa receptor precursor (LDLR-p), and the 90-kDa transferrin receptor (TFR) are shown. (*Right*) The supernatants from the initial precipitation were then subjected to a second immunoprecipitation, with the polyclonal anti-LDL receptor antibody 698-7, and analyzed by NaDodSO₄/polyacrylamide gel electrophoresis and fluorography.

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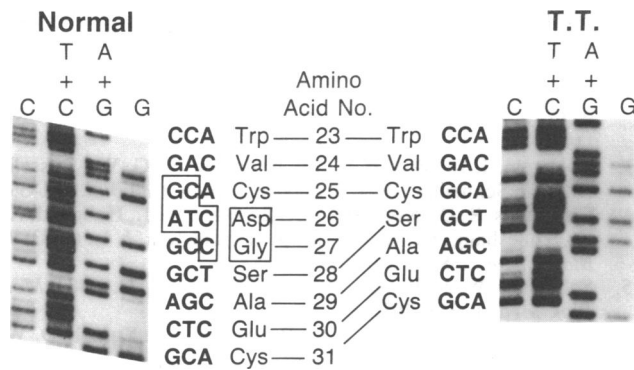*Proc. Natl. Acad. Sci. USA 85 (1988)*   7915

FIG. 5. Partial nucleotide sequences from exon 2 of LDL receptor alleles from a normal subject and FH homozygote TT. Exon 2 of the LDL receptor gene was amplified by using two oligonucleotides: end-labeled PCR-58 and unlabeled PCR-57. Sequence analysis of the antisense strand from a normal subject and from TT was performed according to Maxam and Gilbert (17). Six base pairs of the normal sequence are deleted in TT (CATCGC), resulting in deletion of amino acid residues 26 and 27 (aspartic acid and glycine) in the mature protein. The missing nucleotides and amino acids are boxed.

for the study). This confirmed that TT was homozygous and his mother heterozygous for the mutation.

DISCUSSION

Recent studies (7) demonstrated that deletion of the first cysteine-rich repeat of the LDL receptor binding domain results in a protein that is transported to the cell surface normally and binds LDL with high affinity but is unable to bind IgG-C7, a monoclonal antibody to the LDL receptor. Binding studies using fibroblasts derived from a skin biopsy specimen of a black South African FH homozygote (TT) showed similar binding characteristics: the cells bound LDL with high affinity (though in decreased amounts) but failed to bind IgG-C7. Exon 2, which encodes the first cysteine-rich repeat, was therefore suspected as a likely locus for the LDL receptor gene mutation in this patient.

Haplotype analysis of the LDL receptor gene using 10 restriction fragment length polymorphic sites (18) suggested that TT was homozygous for the mutant LDL receptor allele (data not shown). We then used the recently described gene-amplification technique (8) to selectively amplify and sequence exon 2 of the LDL receptor gene, and we determined the nucleotide sequence of the amplified DNA by the Maxam–Gilbert technique (17). A 6-bp deletion was identified in the 3' half of exon 2. The deletion is predicted to result in the elimination of two amino acids, aspartic acid and glycine, from the LDL receptor protein. The deletion interrupts two flanking codons but preserves a triplet codon for a cysteine residue. This mutation creates a new *Pst* I restriction site in exon 2, which was used to confirm that the patient was homozygous and his mother heterozygous.

FIG. 6. Comparison of the amino acid sequence of the first repeat between the rabbit and the human LDL receptor. The amino acids deleted in TT are boxed. The amino acids conserved between the rabbit and the human are denoted by connecting lines. Cysteine residues are denoted by stars. The one-letter amino acid code translates to the three-letter code as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
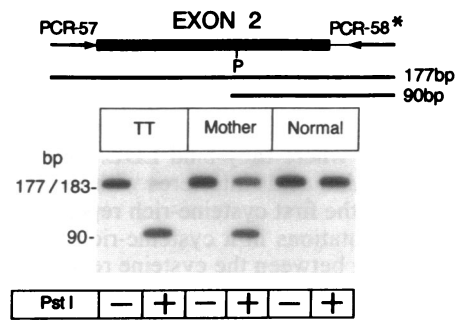

FIG. 7. *Pst* I restriction analysis of TT's mutation. Exon 2 of the LDL receptor gene was amplified by using end-labeled PCR-58 oligonucleotide and unlabeled PCR-57 oligonucleotide. Ten base pairs separate PCR-58 from the 3' end of exon 2. The DNA was purified by electrophoresis in a 6% polyacrylamide gel and ≈100 ng was digested twice with 10 units of *Pst* I restriction endonuclease for 1 hr at 37°C. The DNA was then extracted with phenol/chloroform (1:1) and precipitated with 3 M sodium acetate (pH 5) and ethanol. The samples were subjected to electrophoresis in a 6% polyacrylamide gel. The gel was autoradiographed for 5 min on a XAR-5 film. The first lane reveals a single 177-bp band, corresponding to the undigested DNA from TT. The second lane shows a 90-bp restriction fragment resulting from *Pst* I digestion of DNA from TT. The third lane shows a single band of undigested DNA from the mother of TT. The expected 183- and 177-bp fragments cannot be resolved under these conditions. For the fourth lane, DNA from TT's mother was digested with *Pst* I, revealing an additional 90-bp fragment. The fifth and sixth lanes show undigested and digested DNA, respectively, from a normal control subject (183-bp fragments in both).

The IgG-C7 antibody recognizes the human but not the rabbit LDL receptor protein (2). In an effort to pinpoint the epitope for IgG-C7, the sequences of the human (6) and rabbit (19) receptors were compared (Fig. 6). The amino acids deleted in TT are present in both the human and the rabbit LDL receptor protein, and therefore it is unlikely that the deleted residues are part of the IgG-C7 binding site of the human LDL receptor. More likely, the conformation of the epitope for IgG-C7 is indirectly altered by the deletion of two amino acids. The spacing between the six cysteine residues in each of the seven ligand-binding repeats is highly conserved (6). Previous experiments demonstrated that all the cysteine residues in the receptor protein are involved in intrachain disulfide bonds (20). Analysis of other proteins with similar cysteine-rich repeats suggests that the cysteine bonds form within each repeat (21). Disulfide bonds have been shown to form cotranslationally in the ER by disulfide isomerase (22). The small in-frame deletion in TT shortens the distance between the fourth and fifth cysteine residues in the first repeat and may therefore interfere with proper formation of disulfide bonds within the repeat and hence interfere with correct protein folding.

Processing of the 120-kDa precursor to the 160-kDa mature LDL receptor occurs in the Golgi complex. There, the high-mannose chains of N-linked sugars are trimmed, galactose residues are added to the O-linked core residues, and sialic residues are added prior to transport to the cell surface (3). Numerous studies have shown that the transport of newly synthesized proteins from the ER to the Golgi apparatus is a specific and highly regulated event (23, 24). Proteins undergo a series of covalent modifications while still in the ER, and mutations that interrupt the proper folding of a protein can result in sequestration of the protein in the ER, as has been demonstrated in the study of induced mutations in the influenza hemagglutinin protein (25, 26) and the G glycoprotein of vesicular stomatitis virus (27, 28). The mechanism whereby malfolded proteins are identified is poorly understood but may involve a "gatekeeper" protein (19, 26) that

recognizes these proteins and halts the transport out of the ER.

In TT, the processing of the 120-kDa LDL receptor precursor protein to the mature form is severely retarded, though receptors are transported through the Golgi complex to the cell surface where they bind LDL. Presumably, the deletion of two amino acids interferes with the formation of disulfide bonds in the first cysteine-rich repeat of the receptor. Two other mutations in a cysteine-rich binding repeat change the spacing between the cysteine residues and result in a class II phenotype. In the Watanabe heritable hyperlipidemic (WHHL) rabbit, an animal model of FH, there is a deletion of four amino acids in the third cysteine-rich binding repeat of the LDL receptor protein (19). FH563, an individual with homozygous FH, has a 3-bp deletion in the fifth repeat resulting in the deletion of a single amino acid, glycine (ref. 19 and D. W. Russell, personal communication). Although the deletions in the WHHL rabbit, FH563, and TT do not include any cysteine residues, they alter the spacing between nearby cysteines. As can be seen in Fig. 6 and in ref. 6, the spacing between cysteine residues in each of the seven repeats is rigorously conserved and may be a requirement for proper protein folding.

Deletion of an entire cysteine-rich ligand-binding domain repeat does not interrupt the reading frame and produces a protein that matures normally, as demonstrated by the characterization of two LDL receptor mutant alleles (7, 15). Deletion of the fifth exon, encoding the sixth cysteine-rich repeat, results in a 100-kDa protein that is processed at a normal rate to 140-kDa prior to its appearance on the cell surface (15). Deletion of the second exon, encoding the first repeat, results in a protein that is rapidly transported from the ER to the Golgi complex (7). The results of these studies suggest that in contrast to a deletion of an entire repeat, a small deletion within a cysteine-rich repeat interferes with protein folding, as reflected by the effect on intracellular receptor trafficking.

The prevalence of TT's mutation in the Black South African community has not been determined. Detection of this mutation by *Pst* I restriction analysis provides a convenient assay for diagnostic purposes.

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